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THE EFFECT OF PROSTAGLANDIN E_2 ON THE ARTERIAL BLOOD PRESSURE OF NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

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1 In anaesthetized rats, injection of prostaglandin E_2 (0.5-5.0 μg/kg i.v.) caused a dose-dependent vasodepressor response. The magnitude of response was significantly greater in spontaneously hypertensive rats than in normotensive rats.

2 In spontaneously hypertensive rats, the magnitude of the prostaglandin-induced depressor response was closely correlated with blood pressure. The higher the blood pressure, the greater was the response evoked by prostaglandin.

3 In spontaneously hypertensive rats, a combination of guanethidine plus hydralazine reduced both blood pressure and prostaglandin-induced depressor responses.

4 Spontaneously hypertensive rats and normotensive rats did not differ in the magnitude of their vasodepressor responses to intravenously administered acetylcholine or isoprenaline.

Introduction

Prostaglandin E_2 is known to be a vasodepressor substance (Karim, 1972). Vasodepression is due, at least in part, to a direct action of the prostaglandin on vascular smooth muscle (von Euler & Eliasson, 1967). However, Leach, Armstrong, Germain & Muirhead, (1973) have recently reported that vasodepressor prostaglandins have a special action in spontaneously hypertensive rats (SHR). According to these authors, prostaglandins may cause prolonged vasodepression due to an action mediated by the vagus nerve. This action does not occur in normotensive rats (NTR).

It is not clear why SHRs and NTRs should respond differently to vasodepressor prostaglandins. Indeed, there are very few data on the relative actions of prostaglandins in normotensive and hypertensive subjects. Therefore, in the report that follows, NTRs and SHRs have been compared with respect to their vasodepressor responses to prostaglandin E_2. Prostaglandin E_2 was chosen for study because it is an endogenous renal substance that may be relevant to blood pressure regulation. In addition, it is rapidly metabolized *in vivo* and thus convenient to use in dose-response studies.

Methods

Animals

Two strains of male Wistar rats were used throughout these studies. SHRs were descendants of the Wistar strain bred selectively for hypertension (Okamoto, 1969). NTRs were derived from an inbred strain of Wistar that is normotensive. Both SHRs (denoted Wistar SHR) and NTRs (denoted Wistar MW-3) were obtained commercially (Carworth Farms). All animals ranged in age from 4 to 20 weeks. The exact age at time of testing is indicated in the results section.

Recording of blood pressure

All studies were conducted on intact animals. For acute experiments, animals were anaesthetized (see results section) with a combination of sodium pentobarbitone (20 mg/kg, i.p.) and urethane (500 mg/kg, i.p.). Blood pressure was measured directly via a cannula (polyethylene tubing number 50) inserted into the carotid artery. Blood pressure recordings were obtained with a transducer (Hewlett Packard 1280B) and amplifier

(Hewlett Packard 8805C) connected to a thermal recording system (Hewlett Packard 7754A). Drugs were injected or infused through a cannula (polyethylene tubing number 10) into the femoral vein.

For chronic experiments, blood pressure was measured pneumatically. A tailcuff transducer (Narco Biosystems pneumatic pulse transducer 705-0022) was interfaced with the recording system described above. Animals were conscious but restrained during blood pressure readings. Measurement of blood pressure was facilitated by pre-warming animals in a heated chamber (32-33° C).

In the results section, data on resting blood pressure and on changes in blood pressure are expressed in terms of the systolic values. This option was chosen because pneumatic readings of systolic pressure are easier to obtain and more accurate than pneumatic readings of mean aortic or diastolic pressure. However, sample records of acute experiments were periodically examined. It was found that the systolic values closely paralleled mean aortic and diastolic values.

Drugs

The drugs used were: isoprenaline hydrochloride (Regis Chemical Company), acetylcholine chloride (Calbiochem), prostaglandin E_2 (a gift of the Upjohn Company), guanethidine and hydralazine hydrochloride (gifts of the Ciba Pharmaceutical Company).

In the results section, drug doses are expressed in terms of the base. For all injections, saline (0.154 M NaCl) was the vehicle. For i.v. injections of drugs, a volume of 0.05 ml was used regardless of dose.

Prostaglandin E_2 was obtained in crystalline form. A sample was dissolved in absolute ethanol, and this served as a stock solution. Immediately before testing, aliquots of the stock solution were evaporated to dryness under a stream of air. The residue was resuspended in physiological saline for injection.

Results

Anaesthesia

In a series of preliminary experiments, rats were given α-chloralose. A dose of 40 mg/kg (i.v.) was chosen, because this was the dose that had been used in a previous report (Okamoto, Hazama, Takeda, Nosaka, Fukushima, Yamori, Marsumoto, Haebara, Ichijima & Suzuki, 1966). However, in the hands of the present investigator, this dose of

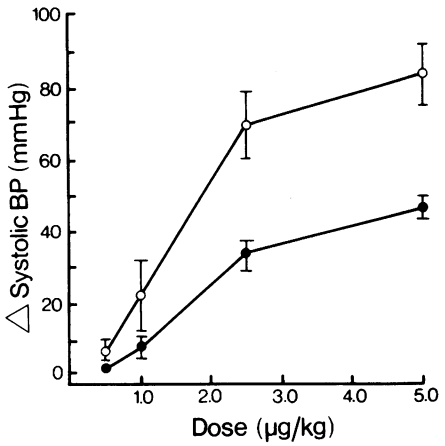

Fig. 1 The change in systolic blood pressure (depressor response) caused by i.v. (femoral vein) administration of prostaglandin E_2 to normotensive (●) and hypertensive (○) rats.

α-chloralose never produced general anaesthesia. Increases in the dose sufficient to produce complete anaesthesia were found to be unworkable, because such increases were invariably accompanied by a notable drop in blood pressure. SHRs fully anaesthetized with α-chloralose had blood pressures within the normotensive range.

In a second series of experiments, SHRs were anaesthetized with sodium pentobarbitone (40-60 mg/kg, i.p.). Again, there was a tendency for the blood pressure of hypertensive animals to fall toward normotensive levels. To prevent the fall in blood pressure, a combination of sodium pentobarbitone (20 mg/kg, i.p.) and urethane (500 mg/kg, i.p.) was used. SHRs treated with this combination maintained a systolic blood pressure of 160 mmHg or greater throughout experimentation.

Blood pressure responses to prostaglandin

Both NTRs and SHRs (12 weeks of age) were tested for their depressor responses to prostaglandin E_2. The drug was administered as a bolus into the femoral vein. Intervals of not less than 15 min were allowed to elapse between drug injections. Depressor responses were assessed by monitoring changes in systolic blood pressure following drug injection. All animals were subjected to full dose-response studies in which the dose of prostaglandin E_2 ranged from 0.5-5.0 μg/kg.

Data comparing NTRs and SHRs are presented in Figure 1. For each point on the figure at least

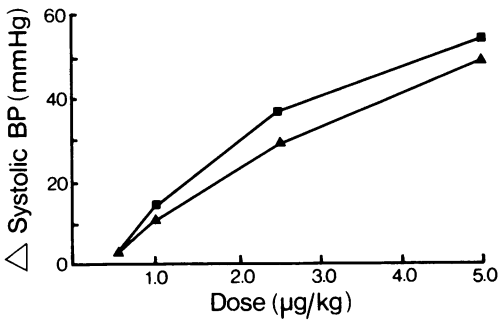

Fig. 2 The change in systolic blood pressure (depressor response) caused by i.v. (femoral vein) administration of prostaglandin E_2 to normotensive Long-Evans (■) and Sprague-Dawley (▲) rats.

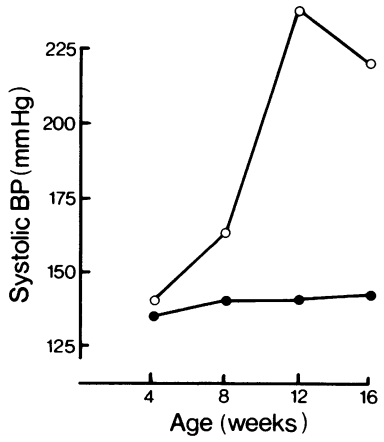

Fig. 3 The systolic blood pressure of normotensive (●) and hypertensive (○) rats of various ages.

five animals were tested; furthermore, each animal was given each dose of drug at least twice. There was a marked difference between NTRs and SHRs in terms of their responses to prostaglandin E_2. SHRs experienced a greater fall in blood pressure. The difference between NTRs and SHRs was highly statistically significant at doses of 2.5 μg/kg and greater ($P < 0.001$).

Conceivably, the different responses shown by NTRs and SHRs could be related to differences in species rather than differences in blood pressures. This possibility was checked by testing two additional strains of normotensive rats. Sprague-Dawley and Long-Evans (12 weeks of age) were the strains chosen for study. Figure 2 shows dose-response data obtained with these animals. The protocol used in obtaining the data was identical to that mentioned in relation to Figure 1. It was found that normotensive Sprague-Dawley and Long-Evans rats responded to prostaglandin E_2 in a manner similar to that of normotensive Wistar rats.

The possibility that the different responses by NTRs and SHRs could be related to differences in blood pressure was tested in two ways. In the first test, NTRs and SHRs of various ages (4, 8, 12 and 16 weeks; group n at least 3) were examined in terms of their systolic blood pressure, and their depressor responses to prostaglandin E_2. For examination of blood pressure, animals were anaesthetized with sodium pentobarbitone and urethane, and cannulae were installed in the right carotid artery. Arterial pressure readings were taken 30 min after completion of surgery. The data are presented in Figure 3. As expected, NTRs showed little increase in blood pressure from 4-16 weeks, but SHRs showed a marked increase. The same animals were then examined for their dose-response characteristics to injected prostaglandin E_2. The protocol used in obtaining the data was identical to that mentioned in relation to Figure 1. For NTRs (Fig. 4a), there was little change in responsiveness from 4-12 weeks. There was no apparent justification for pursuing the study of 16 week old NTRs. For SHRs (Fig. 4b), there was a notable increase in responsiveness with age, just as there had been an increase in blood pressure (Figure 3).

In a second and perhaps more appropriate test for the relationship between blood pressure and responsiveness to prostaglandin E_2, an analysis of correlation was performed. All animals used in the preceding study were included. The correlation sought was that of resting systolic blood pressure versus the change in systolic blood pressure (depressor response) caused by prostaglandin E_2 (5 μg/kg). The data are presented in Fig. 5 and show that there was a high statistically significant correlation between blood pressure and depressor responses ($r = 0.922$; $P < 0.001$). The higher the systolic blood pressure, the greater was the response evoked by prostaglandin E_2.

Anti-hypertensive drug treatment

A question naturally arises as to whether increased responsiveness to prostaglandin E_2 is a cause of high blood pressure or a result of high blood pressure. Two simultaneous experiments were undertaken to answer this question. In the first, 4-week-old SHRs (group n=3) were given drinking water that contained guanethidine and hydralazine (250 mg each per litre). The animals were maintained on this regimen for 10 weeks. The purpose of the experiment was to prevent the

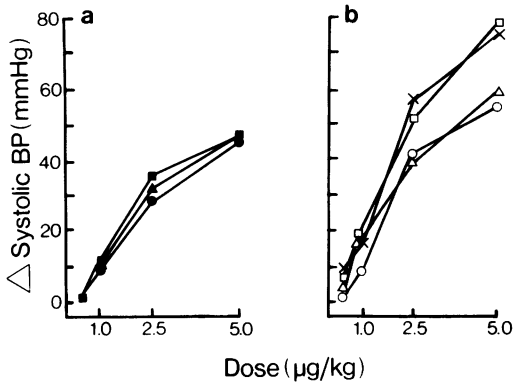

Fig. 4 The change in systolic blood pressure (depressor response) caused by i.v. (femoral vein) administration of prostaglandin E_2 to normotensive (a) and hypertensive (b) rats of various ages. See Fig. 5 for explanation of symbols.

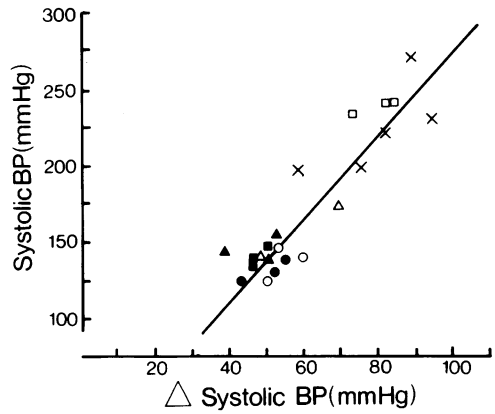

Fig. 5 A simultaneous plot (correlation) of resting systolic blood pressure versus the change in systolic blood pressure (depressor response) caused by administration of prostaglandin E_2 to normotensive (closed symbols) and hypertensive (open symbols) rats. Age of rats: (●○) 4 weeks; (▲△) 8 weeks; (■□) 12 weeks; (x) 16 weeks. Number of animals in the experiment ($n = 22$); correlation coefficient ($r = 0.922$).

development of hypertension. In the second experiment, 10-week-old SHRs (group n=3) were given drinking water with guanethidine and hydralazine (250 mg each per litre). The animals were maintained on this regimen for 6 weeks. The purpose of the experiment was to reverse the hypertension that had already developed. In both experiments, suitable groups (n=3) of control SHRs were maintained *ad libitum* on tap water. In addition, all groups were subjected to weekly readings of blood pressure (pneumatic) to ensure that drug treatment had produced the desired effect.

The results from both experiments are presented in Figure 6. The protocol used in obtaining the data was identical to that mentioned in relation to Figure 1. Part (a) of the figure depicts responses to prostaglandin E_2 by one group of control SHRs (average systolic blood pressure = 198 mmHg), and by SHRs in which hypertension was prevented (average systolic blood pressure = 97 mmHg). Figure 6b depicts responses to prostaglandin E_2 by the second group of control SHRs (average systolic blood pressure = 205 mmHg), and by SHRs in which hypertension was reversed (average systolic blood pressure = 112 mmHg). In both experiments, untreated SHRs were highly responsive to prostaglandin E_2. On the other hand, treated SHRs in which hypertension had been prevented (Fig. 6a) or reversed (Fig. 6b) had reduced responses to prostaglandin E_2.

Blood pressure responses to acetylcholine and to isoprenaline

Both NTRs and SHRs were tested for their depressor responses to acetylcholine and to isoprenaline. Drugs were administered as a bolus via the femoral vein. Intervals of not less than 3 min were allowed to elapse between drug injections. All animals were subjected to dose-response studies in which the dose of administered drug ranged from 0.01-3.3 μg/kg. Results comparing NTRs and SHRs with respect to their reactivity to depressor agents are shown in Figure 7. Part (a) of the figure depicts responses to acetylcholine; part (b), responses to isoprenaline. For each point on the figure, at least five animals were tested; furthermore, each animal was given each dose of drug at least three times.

As the data show, there was little difference between normotensive and hypertensive animals in terms of their depressor responses to acetylcholine and to isoprenaline. Although there was a tendency for NTRs to be more reactive to high doses of drug, this tendency was not statistically significant. On the other hand, the difference in systolic blood pressure between NTRs and SHRs used in the dose-response studies was significantly different ($P < 0.01$). The average systolic pressures for NTRs and SHRs were, respectively, 133 mmHg and 185 mmHg.

Resting blood pressure

In all dose-response studies involving vasodepressor substances (prostaglandin E_2, acetylcholine, isoprenaline), blood pressure was monitored throughout the experiment. In no case was there a

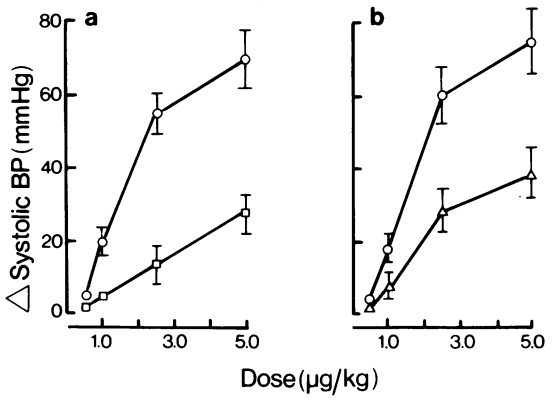

Fig. 6 The change in systolic blood pressure (depressor response) caused by administration of prostaglandin E_2: (a) responses by control hypertensive rats (○) and by rats in which hypertension was prevented (□); (b) responses by a second group of control hypertensive rats (○) and by rats in which hypertension was reversed (△).

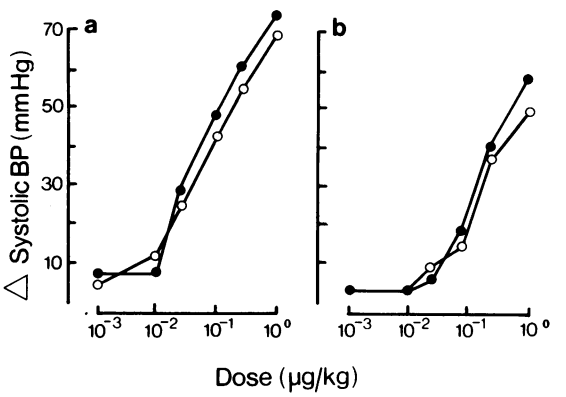

Fig. 7 The change in systolic blood pressure (depressor response) caused by i.v. (femoral vein) administration of acetylcholine (a) and isoprenaline (b) to normotensive (●) and hypertensive (○) rats.

persistent fall in blood pressure (systolic, mean aortic, or diastolic) over time. In dose-response studies with acetylcholine and isoprenaline, resting blood pressure remained relatively constant. In studies with prostaglandin E_2, there occasionally occurred an increase in systolic pressure. This was not studied in detail, but it appeared to be due to an increased stroke volume and thus, presumably, an increased cardiac output.

Discussion

Leach *et al.* (1973) have reported a special action of vasodepressor prostaglandins in SHRs. According to these authors, either prostaglandin E_2 or A_2, when administered intravenously as a bolus, causes a prolonged fall in arterial blood pressure. The phenomenon is evident only when prostaglandins are administered according to a rigid dose and time schedule. Furthermore, the phenomenon is observed only in SHRs; a prolonged fall in blood pressure does not occur in NTRs. No explanation for the differential responses of NTRs and SHRs has been proposed.

The aforementioned study points to the necessity for investigations on the comparative pharmacology of prostaglandins in NTRs and in SHRs. This necessity is heightened by two hypotheses that are currently the subject of much research. According to one hypothesis, vasodepressor prostaglandins play a central role in blood pressure regulation. In fact, it has been proposed that a deficiency of prostaglandins could be a causative factor in hypertension (e.g. Lee, 1967). In light of this hypothesis, it would be helpful to understand both the action and metabolism of prostaglandins in suitably matched normotensive and hypertensive subjects. According to a second hypothesis, the SHR is an ideal animal model for human essential hypertension (Okamoto, 1972). If this hypothesis is true, then the study of prostaglandin action in SHRs may contribute to an understanding of prostaglandin action in man. Thus, there appears to be ample justification for comparison of the action of prostaglandin E_2 in NTRs and in SHRs.

In this paper, prostaglandin E_2 was administered intravenously to anaesthetized rats. This technique is in keeping with that described in many reports on prostaglandin action; however, there are both advantages and disadvantages to the intravenous route. The major disadvantage is that prostaglandin E_2 is rapidly inactivated in the lungs (Samuelsson, 1964; Anggard & Samuelsson, 1964). This drawback can be overcome by administering somewhat larger doses. The major advantage to the intravenous route is that the drug is distributed to all vascular beds. Since this study was undertaken as an initial exploration of comparative drug action, it was desirable to achieve maximum regional distribution.

It has been found that the vasodepressor action of prostaglandin E_2 is significantly greater in SHRs than in NTRs. The difference in responsiveness to prostaglandin E_2 does not reflect differences between strains because three strains of normotensive rats (Wistar, Sprague-Dawley and Long-Evans) were tested. All three had similar dose-response characteristics to prostaglandin E_2, and

all three differed significantly from SHRs. In addition, the difference between NTRs and SHRs in response to prostaglandin E_2 was not a generalized characteristic of depressor substances. NTRs and SHRs did not differ in their responses to acetylcholine or to isoprenaline (Okamoto *et al.*, 1966, Leach *et al.*, 1973; this paper).

The increased responsiveness of SHRs appears to be closely correlated with blood pressure. This conclusion is supported by several observations: (1) As SHRs age, both their blood pressure and their responsiveness to prostaglandin E_2 increase. As NTRs age, neither their blood pressure nor their responsiveness to prostaglandin E_2 changes. (2) Among hypertensive rats, there is a highly statistically significant correlation between blood pressure and prostaglandin E_2-induced vasodepression. The higher the blood pressure, the greater the response evoked by prostaglandin. (3) When the development of hypertension in SHRs is prevented, increased responsiveness to prostaglandin E_2 does not develop. (4) When hypertension in SHRs is reversed, increased responsiveness to prostaglandin E_2 is lost.

These data indicate strongly that there is a relationship between blood pressure in SHRs and responsiveness to prostaglandin E_2. However, the data do not show that the cause of hypertension is also the cause of hyper-responsiveness to prostaglandins. Additional data bearing on the pathology of SHRs and the pharmacology of prostaglandins are necessary before any links can be established. Furthermore, the results should not be interpreted to mean that SHRs differ fundamentally from NTRs in response to prostaglandins. The data indicate only that there is a difference in the magnitude of response, not that there is a difference in the mechanism of response.

In view of the fact that the cause of hypertension in SHRs had not been established, it is difficult to explain why SHRs should be hyperresponsive to prostaglandin E_2. However, there is at least one line of investigation worth pursuing. It has been a universal finding that neuroactive anti-hypertensive drugs (e.g. α-methyl tyrosine, α-methyl dopa, reserpine, etc.) produce a greater drop in blood pressure in hypertensive than in normotensive subjects. SHRs are no exception to this rule (Laverty & Robertson, 1967; Freis, Ragan, Pillsbury & Matthews, 1972). It has also been demonstrated that prostaglandins of the E series can act to depress neurogenic release of noradrenaline (cf. Hedqvist, 1973). Hence, it is possible that prostaglandin E_2 evokes a relatively greater response in SHRs as compared to NTRs by virtue of diminishing sympathetic activity. This possibility is under investigation.

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